*INCREASE IN RAT BRAIN TYROSINE HYDROXYLASE ACTIVITY PRODUCED BY ELECTROCONVULSIVE SHOCK**

By Jose M. Musacchio, Louis Julou,† Seymour S. Kety,‡ and Jacques Glowinski

DEPARTMENT OF PHARMACOLOGY, NEW YORK UNIVERSITY SCHOOL OF MEDICINE, AND UNITÉ DE NEUROPHARMACOLOGIE BIOCHIMIQUE, LABORATOIRE DE NEUROPHYSIOLOGIE GÉNÉRALE, COLLÈGE DE FRANCE, PARIS

Communicated May 22, 1969

Abstract.—A sustained increase in the turnover of norepinephrine coupled with an increased concentration of that amine has, in a previous study, been observed in the brains of rats exposed to electroconvulsive shock twice daily for one week. The phenomenon has been further examined by studying the effects of a similar regimen of electroconvulsive shock upon tyrosine hydroxylase in the brain, since that enzyme appears to be rate-limiting in norepinephrine synthesis. A small (15%) but significant increase in the whole brain enzyme activity was found in the electroshock-treated animals. A significant increase of tyrosine hydroxylase activity was found in the brainstem (24%) and cortex (20%) of such animals.

Chronic electroconvulsive shock induces a sustained increase in the synthesis and utilization of norepinephrine in different parts of the rat central nervous system:[1] (*a*) brainstem and mesencephalon, (*b*) telencephalon and diencephalon, and (*c*) spinal cord. Since these changes in norepinephrine are observed 24 hours after the end of the electroconvulsive shock treatment, it was decided to examine the possibility that the acceleration of norepinephrine synthesis could be related to an increase in the activity of tyrosine hydroxylase which appears to be the rate-limiting enzyme in norepinephrine synthesis.

Methods.—Male albino rats (Charles River Strain), approximately 275 gm in weight, were randomly divided into two groups: One of these groups was subjected to electroconvulsive shock for 7 days by the method employed previously;[1] the other was treated similarly, even to application of electrodes, except that the current was not turned on. The rats were decapitated 24 hr after the last period of electroconvulsive shock or "sham shock." The whole brain was quickly removed and frozen or dissected in the cold. The following regions were isolated: (*a*) brainstem and mesencephalon, (*b*) hypothalamus, (*c*) thalamus, (*d*) cortex, and (*e*) caudate nuclei. The tissues were then frozen and kept in dry ice until tyrosine hydroxylase was determined, between 36 hr and 3 days after decapitation. Freezing and storage for 3 days was found not to alter the activity of rat brain tyrosine hydroxylase.

Partial purification and determination of tyrosine hydroxylase: All the samples in experiment 1 (whole brain) and the samples corresponding to the same brain region (experimental and control) in experiment 2 were processed at one time, following a random order to minimize time effects. The frozen tissues were weighed and homogenized in 10 ml of ice-cold distilled water; larger amounts of tissue (cortex and the whole brain) were homogenized in 20 ml. All procedures were performed at 4°C unless otherwise stated. The homogenate was transferred to a polycarbonate centrifuge tube, balanced by the addition of water, and centrifuged at 100,000 × *g* for 1 hr. The supernatant was transferred to a 50-ml plastic centrifuge tube, and 7 ml (14 ml for the cortex and whole brain) of a saturated solution of ammonium sulfate were added while the mixture was being

stirred in order to precipitate tyrosine hydroxylase. The saturated ammonium sulfate solution was prepared in 0.02 *M* potassium phosphate (monobasic) and the final pH was adjusted to 7.0 with concentrated KOH. The mixture was allowed to equilibrate for about 20 min and was then centrifuged for 20 min at 10,000 × *g*. The supernatant was discarded and the walls of the test tubes were carefully blotted with tissue paper; the precipitate was then dissolved in 1 ml of 0.005 *M* Tris–HCl buffer pH 7.0, which was then transferred with a Pasteur pipette to a graduated centrifuge tube where the final volume was measured. Two aliquots of 0.4 ml were used for tyrosine hydroxylase measurements in duplicate. Tyrosine hydroxylase was determined by the method of Nagatsu *et al.*[2] with minor modifications. The incubation mixture contained the following components: 0.4 ml of tissue extract; 0.1 ml of 1 *M* Tris–maleate–KOH buffer, pH 5.9; tyrosine 3,5-H^3, 200 mμM; NSD 1055, 0.1 μM; ferrous ammonium sulfate, 0.5 μM; 6,7-dimethyl-5,6,7,8-tetrahydropterine ($DMPH_4$), 1 μM in 0.1 ml of 1 *M* mercaptoethanol; water up to 1 ml. The incubation tubes were kept in an ice bath while the different components were added. The reaction was started by the addition of the $DMPH_4$–mercaptoethanol mixture after a preincubation of 4 min at 25°C. The tubes were incubated exactly 15 min at the same temperature, and the reaction was stopped by the addition of 0.1 ml of 30% trichloroacetic acid. The tubes were centrifuged, and a 0.5-ml aliquot of the supernatant was directly applied to a 4 × 0.6-cm column of H^+ Dowex (50 × 4). The column was washed with 1 ml of water, and 0.2 ml of the mixture of effluent and wash were assayed for radioactivity in a liquid scintillation spectrometer. The tyrosine hydroxylase activity is linear for 30 min and is proportional to the amount of brain extract.

Results and Discussion.—The results of the determinations of tyrosine hydroxylase are shown in Table 1. There is a small (15%) but significant increase in the whole brain enzyme activity (expt. 1) of the electroshock-treated animals. Regional studies (expt. 2) demonstrated a significant increase of tyrosine hydroxylase activity in the brainstem (24%) and cortex (20%) of the shocked animals; no differences were found in the caudate and hypothalamus. The activity in the thalamus was below the sensitivity of the method. There are

TABLE 1. *Effect of electroconvulsive shock on rat brain tyrosine hydroxylase.*

	Control	Experimental	Δ%
	(mμM/hr/gm wet tissue ± SEM)		
Experiment 1			
Whole brain	59.2 ± 1.93	68.3 ± 1.78*	+15.3
Experiment 2			
Brain stem and mesencephalon	42.7 ± 2.12	53.2 ± 2.21*	+24.5
Cortex	40.3 ± 2.93	48.6 ± 2.87†	+20.5
Hypothalamus	101.7 ± 5.43	98.0 ± 3.78	− 3.7
Caudate nuclei	496.0 ± 24.0	534.0 ± 19.9	+ 7.6
	Control	Experimental	Δ%
	(mμM/hr/region ± SEM)		
Experiment 1			
Whole brain	114.12 ± 4.05	130.12 ± 2.59*	+14.0
Experiment 2			
Brain stem and mesencephalon	15.89 ± 0.75	19.31 ± 0.81*	+21.5
Cortex	32.18 ± 2.50	40.09 ± 2.31†	+24.5
Hypothalamus	8.59 ± 0.55	9.04 ± 0.51	+ 5.2
Caudate nuclei	52.15 ± 2.72	52.33 ± 1.79	—
Total	108.81	120.77	+10.9

Each result is the average for 10 animals in expt. 1 and 12 animals in expt. 2.
Δ%, change (%) from control value.
* $p < 0.01$.
† $p < 0.05$.

slight variations in the percentage increase of tyrosine hydroxylase when the results of enzyme activity per gram of wet tissue are compared with the activity per region, but in both cases the increases are significant. Tyrosine hydroxylase activity was determined after partial purification of the enzyme by centrifugation and precipitation with ammonium sulfate: the enzyme activity measured is free of the effects of endogenous tyrosine, endogenous pteridine co-factor, and the pteridine regenerating system. The increase in tyrosine hydroxylase activity is therefore due to an increase in enzyme—by increased synthesis or reduced degradation—or to activation of preformed enzyme.

There are experiments that strongly suggest that tyrosine hydroxylase is an inducible enzyme: Weiner and Rabadjija[3] have described a puromycin-sensitive increase of norepinephrine synthesis following nerve stimulation in the guinea pig vas deferens. It is not possible to establish with available experimental data whether the increase in tyrosine hydroxylase activity observed in the brain stem and cortex of electroshocked rats is due to an induction of tyrosine hydroxylase, but the persistence of the changes observed and the inducibility of tyrosine hydroxylase are suggestive of such a possibility. Since prolonged or chronic stress of other types is associated with an increase in rate of turnover of norepinephrine in the central nervous system,[4] it is possible that an increase in tyrosine hydroxylase activity in certain parts of the brain may represent an adaptive response to stress.

We wish to thank Robert Wurtzburger for his valuable assistance in the determination of tyrosine hydroxylase.

* This investigation was supported in part by NIH grant HE 10333 and by La Société des Usines Chimiques Rhone-Poulenc.

† Laboratoires de Recherches de La Société des Usines Chimiques Rhone-Poulenc.

‡ Department of Psychiatry, Harvard Medical School at Massachusetts General Hospital, Boston, Massachusetts.

[1] Kety, S. S., F. Javoy, A. Thierry, L. Julou, and J. Glowinski, these PROCEEDINGS, **58**, 1249 (1967).

[2] Nagatsu, T., M. Levitt, and S. Udenfriend, *Anal. Biochem.*, **9**, 122 (1964).

[3] Weiner, N., and M. Rabadjija, *J. Pharmacol. Exptl. Therap.*, **164**, 103 (1968).

[4] Thierry, A., F. Javoy, J. Glowinski, and S. S. Kety, *J. Pharmacol. Exptl. Therap.*, **163**, 163 (1968).